Prevalence of sheep infected with classical scrapie in Great Britain: integrating multiple sources of surveillance data for 2002

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Electronic appendix A Demographic data

This appendix presents details of the demographic data used when estimating the prevalence of sheep infected with classical scrapie in Great Britain (GB).

A.1 Survival probabilities

The frequency of animals over one year old (table A.1) was derived from the number of animals of each age class as reported for 4998 flocks in an anonymous postal survey conducted in 2002 (Sivam *et al*. 2003). The number of lambs (i.e. animals under one year old) was calculated assuming an average of 1.3 lambs was produced per ewe (Webb *et al*. 2001; Pollott & Stone 2006).

The probability of survival to age class a (s_a) was estimated from the postal survey data by computing the number of animals surviving to be in the age class or older divided by the total number of animals in the data set (Collett 1994), so that,

$$s_a = \frac{\sum_{i=a}^{a_{\max}} n_i}{\sum_{i=1}^{a_{\max}} n_i}, \qquad \text{(A.1)}$$

where n_a is the number of animals in age class a and $a_{\max}$ is the maximum age class (table A.1).

A.2 Prion protein (PrP) genotype structure of the national flock

The frequency of PrP genotypes in the GB national flock was estimated using data from two sources (table A.2). The first source was sheep sampled between October 2001 and January 2003 as part of the National Scrapie Plan for GB (NSP; Eglin *et al*. 2005). This incorporated results for 38 non-rare breeds for which there were at least 500 confirmed genotypes from at least ten flocks. The second source was sheep from around 60 flocks (half scrapie-affected, half scrapie-free) sampled as part of a case-control study run by the Institute for Animal Health (IAH; Baylis *et al*. 2004).

Table A.1. *Age data from the 2002 postal survey and estimated survival probabilities.*

age class	1	2	3	4	5	6	7	8	9
age (years)	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	>8
no. sheep†	2246680	336524	366122	377320	342216	202957	71236	21793	10047
probability of survival (s_a)	1.000	0.435	0.350	0.258	0.163	0.077	0.026	0.008	0.003

† the number of sheep in age classes 2-9 was derived from farmers' responses to 2002 postal survey; the number of sheep in age class 1 was estimated assuming 1.3 lambs are produced per ewe

Table A.2. *Frequencies of PrP genotypes for sheep sampled as part of the National Scrapie Plan for GB (NSP) or a case-control study run by the Institute for Animal Health (IAH).*

PrP genotype	NSP		IAH	
	number	%	number	%
ARR/ARR	70388	26.28	2959	21.28
ARR/AHQ	19625	7.33	778	5.60
ARR/ARH	17309	6.46	291	2.09
ARR/ARQ	71735	26.78	3890	27.98
AHQ/AHQ	3965	1.48	264	1.90
AHQ/ARH	2296	0.86	41	0.29
AHQ/ARQ	13790	5.15	875	6.29
ARH/ARH	10417	3.89	222	1.60
ARH/ARQ	8016	2.99	222	1.60
ARQ/ARQ	30712	11.47	1695	12.19
ARR/VRQ	9031	3.37	1334	9.60
AHQ/VRQ	2166	0.81	347	2.50
ARH/VRQ	1704	0.64	41	0.29
ARQ/VRQ	6016	2.25	819	5.89
VRQ/VRQ	669	0.25	125	0.90
total	267839	-	13903	-

The number of sheep of genotype j in a birth cohort, B_j, was estimated to be,

$$B_j = g_j N_L, \tag{A.2}$$

where g_j is the proportion of the GB national flock carrying genotype j (table A.2) and N_L is the number of lambs born in GB in 2002 (16154227 lambs; obtained from the June agricultural survey for 2002).

A.3 Proportion of uninfected animals found dead on farm

The frequency of animals found dead or sent to slaughter was collected for 89 purebred flocks between 2004 and 2006[1] as part of a study investigating possible associations between PrP genotype and health and production traits. Ten important sheep breeds from all sectors of the British sheep industry were included in the study. These data were used to estimate the proportion of uninfected animals found dead on farm in each age class (table A.3).

[1] Unpublished data kindly provided by Rachel Eglin and Charlotte Cook (Veterinary Laboratories Agency).

Table A.3. *Frequency of sheep found dead on farm and sent to slaughter for 89 purebred flocks in GB and parameters estimated from them*.

age class *a*	1	2	3	4	5	6	7	8	9
age (years)	0-1[†]	1-2	2-3	3-4	4-5	5-6	6-7	7-8	>8
no. found dead	-	69	112	108	108	80	73	28	25
no. culled	-	26	222	391	435	464	424	237	180
proportion (%) of uninfected animals found dead on farm (η_a)	-	72.63	33.53	21.64	19.89	14.71	14.69	10.57	12.20

[†] only animals over 18 months of age were sampled in the 2002 fallen stock and abattoir surveys

Electronic appendix B Age-at-onset parameters

Because of the small number of cases reported for most PrP genotypes in a single year, estimates for the age-at-onset parameters (μ_j and σ_j) were obtained using data on the age-at-onset of cases reported between July 1998 and December 2005. For some PrP genotypes, however, there were still too few cases and, hence, it was necessary to group certain of them together when estimating parameters (figure B.1; table B.1). These groupings were based on a descriptive analysis of the number and age distribution of reported cases for each PrP genotype.

The age-at-onset parameters were estimated using a similar approach to that presented in the main paper, so that the expected number of reported cases, R_{ja}, of genotype j in age class a (comprising animals between a-1 and a years of age) is given by,

$$R_{ja} = s_a B_j \theta_j \int_{a-1}^{a} f_j(u)\,\mathrm{d}u, \tag{B.1}$$

where s_a is the probability of surviving to age class a (table A.1), B_j is the number of sheep of genotype j in a birth cohort, θ_j is a composite parameter reflecting the risk of infection in genotype j and under-ascertainment of cases (treated as a nuisance parameter during estimation) and f_j is the probability density function for the log-normal incubation period distribution (with genotype-specific parameters, μ_j and σ_j). The number of reported cases was assumed to follow a Poisson distribution with the mean given by the expected number of reported cases, for which the log-likelihood (l_{RC}) is,

$$l_{RC} = \sum_j \sum_a \left\{ -R_{ja} + X_{ja} \log(R_{ja}) - \log(X_{ja}!) \right\}, \tag{B.2}$$

where X_{ja} and R_{ja} are the observed and expected number of reported cases of genotype j in age class a, respectively. Parameters estimates were obtained by determining the values which maximise the log-likelihood, l_{RC}, while 95% confidence intervals were computed using the profile log-likelihood (see, for example, Pawitan 2001). The parameters were estimated using both the IAH and NSP estimates for the frequency of PrP genotypes in the GB national flock (see appendix A).

There was reasonable agreement between the observed and expected number of reported cases in all PrP genotypes, or groups of PrP genotypes (figure B.1). Estimates for the age-at-onset parameters (μ_j and σ_j) were independent of those used for the frequency of PrP genotypes in the GB national flock, except for the ARR/VRQ genotype (table B.1). Moreover, the estimates for all PrP genotypes were higher than those derived previously (Gubbins & Roden 2006). This difference is most likely because previous estimates for the age-at-onset parameters ignored the impact of survivorship on the onset of disease. Consequently, they ignored those infected animals that were culled before they developed clinical disease.

In the analyses presented in the main paper, the maximum likelihood estimates for age-at-onset parameters were used for all PrP genotypes, except ARR/VRQ. For this genotype the lower 95% confidence limits were used instead, to avoid unrealistically high estimates for the risk of infection, such that all animals of this genotype would become infected.

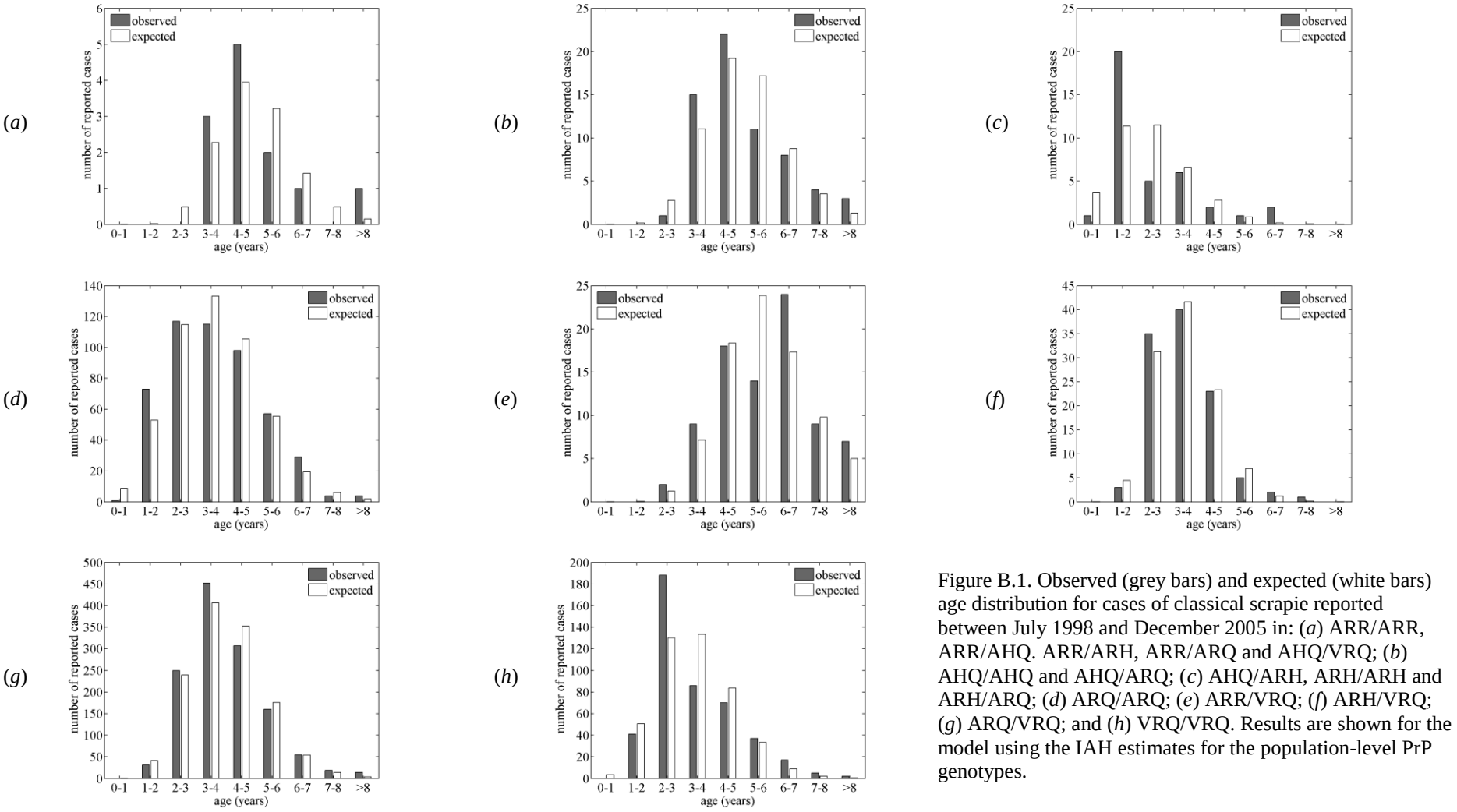

Figure B.1. Observed (grey bars) and expected (white bars) age distribution for cases of classical scrapie reported between July 1998 and December 2005 in: (*a*) ARR/ARR, ARR/AHQ. ARR/ARH, ARR/ARQ and AHQ/VRQ; (*b*) AHQ/AHQ and AHQ/ARQ; (*c*) AHQ/ARH, ARH/ARH and ARH/ARQ; (*d*) ARQ/ARQ; (*e*) ARR/VRQ; (*f*) ARH/VRQ; (*g*) ARQ/VRQ; and (*h*) VRQ/VRQ. Results are shown for the model using the IAH estimates for the population-level PrP genotypes.

Table B.1. *Maximum likelihood estimates (MLEs) and 95% confidence limits (CLs) for the age-at-onset parameters for each PrP genotype and their dependence on the estimates used for the frequency of PrP genotypes in the GB national flock*.

PrP genotype[†]	IAH frequencies						NSP frequencies					
	age-at-onset parameter (μ_j)			age-at-onset parameter (σ_j)			age-at-onset parameter (μ_j)			age-at-onset parameter (σ_j)		
	MLE	95% CL		MLE	95% CL		MLE	95% CL		MLE	95% CL	
		lower	upper		lower	upper		lower	upper		lower	upper
ARR/ARR[a‡]	2.21	1.66	8.25	0.40	0.21	1.48	2.21	1.66	8.33	0.40	0.21	1.49
ARR/AHQ[a]	2.21	1.66	8.25	0.40	0.21	1.48	2.21	1.66	8.33	0.40	0.21	1.49
ARR/ARH[a‡]	2.21	1.66	8.25	0.40	0.21	1.48	2.21	1.66	8.33	0.40	0.21	1.49
ARR/ARQ[a]	2.21	1.66	8.25	0.40	0.21	1.48	2.21	1.66	8.33	0.40	0.21	1.49
AHQ/AHQ[b]	2.65	1.99	5.05	0.50	0.32	0.94	2.65	1.99	5.00	0.50	0.32	0.93
AHQ/ARH[c‡]	1.16	0.87	2.09	0.60	0.44	0.98	1.16	0.87	2.09	0.60	0.44	0.98
AHQ/ARQ[b]	2.65	1.99	5.05	0.50	0.32	0.94	2.65	1.99	5.00	0.50	0.32	0.93
ARH/ARH[c]	1.16	0.87	2.09	0.60	0.44	0.98	1.16	0.87	2.09	0.60	0.44	0.98
ARH/ARQ[c]	1.16	0.87	2.09	0.60	0.44	0.98	1.16	0.87	2.09	0.60	0.44	0.98
ARQ/ARQ	2.58	2.11	3.51	0.80	0.67	1.00	2.58	2.11	3.51	0.80	0.67	1.00
ARR/VRQ	3.88	2.48	4.19	0.61	0.37	0.70	3.56	2.46	3.83	0.56	0.37	0.65
AHQ/VRQ[a]	2.21	1.66	8.25	0.40	0.21	1.48	2.21	1.66	8.33	0.40	0.21	1.49
ARH/VRQ	1.43	1.32	1.62	0.35	0.29	0.45	1.43	1.32	1.62	0.35	0.29	0.45
ARQ/VRQ	1.89	1.79	2.02	0.47	0.44	0.52	1.89	1.79	2.02	0.47	0.44	0.52
VRQ/VRQ	1.65	1.51	1.86	0.53	0.47	0.62	1.65	1.51	1.86	0.53	0.47	0.62
goodness-of-fit[*]	AIC=475.05; χ^2=201.36; df=48						AIC=475.63; χ^2=201.99; df=48					

[†] data for PrP genotypes with a common superscripted letter were combined when estimating the age-at-onset parameters

[‡] there were no reported cases for these PrP genotypes

[*] AIC: Akaike information criterion; df: degrees of freedom

Electronic appendix C Sensitivity and specificity of diagnostic tests

Animals sampled in the fallen stock (del Rio Vilas *et al*. 2005) or abattoir (Elliott *et al*. 2005) surveys were screened using either the BioRad Platelia ELISA (Grassi *et al*. 2001) or the Prionics Check Western blot (Schaller *et al*. 1999). Data on the sensitivity of these diagnostic tests are available for samples taken from confirmed clinical cases, which indicate that the tests are highly sensitive (>99.5%; Philipp *et al.* 2005). Equivalent data are not, however, available for samples taken from infected, but preclinical animals. It was assumed that an infected animal would be detected provided it was at an appropriate stage of the incubation period. This was expressed in terms of the proportion of the incubation period (δ) during which the diagnostic test is able to detect disease-associated prion protein (PrP^{Sc}; taken to be an indicator of infectivity) in preclinical animals, which was assumed to be the same for both tests.

Pathogenesis experiments provide some information on the preclinical detection proportion (δ), but are limited in terms of the number of animals and PrP genotypes tested. In one study PrP^{Sc} was detected by immunohistochemistry (IHC) prior to the onset of clinical disease in VRQ/VRQ animals at 10 (one positive out of two tested), 14 (2/2), 17 (1/1) and 21 (1/1) months of age in a flock in which the age-at-onset in this genotype was around 24 months (van Keulen *et al*. 2002). In a second study PrP^{Sc} was detected by IHC prior to the onset of clinical disease in ARQ/ARQ animals at 21 (2/2) months of age in a flock of Suffolk sheep in which the mean age-at-onset in this genotype was 28 months (Jeffrey *et al*. 2001). These data suggest that δ is likely to lie somewhere between 25% and 50%.

The specificity of the diagnostic tests has been evaluated using scrapie-free sheep from New Zealand (Philipp *et al*. 2005). Samples from healthy adult sheep of a range of breeds and PrP genotypes were tested, none of which produced a (false) positive result. This yielded an estimate for the specificity of 100% (lower 95% confidence limit: 99.6%) and, consequently, the tests were assumed to be 100% specific.

Electronic appendix D Estimates for the risk of infection in each PrP genotype

Table D.1. *Maximum likelihood estimates (MLEs) and 95% confidence limits (CLs) for the baseline risk of infection and the relative risk of infection in each PrP genotype and their dependence on the estimates used for the frequency of PrP genotypes in the GB national flock and the preclinical detection period (δ).*

	IAH frequencies						NSP frequencies					
	δ=25%			δ=50%			δ=25%			δ=50%		
	MLE	95% CL		MLE	95% CL		MLE	95% CL		MLE	95% CL	
		lower	upper		lower	upper		lower	upper		lower	upper
baseline risk (ϕ)	0.120	0.081	0.174	0.042	0.028	0.061	0.207	0.141	0.295	0.076	0.052	0.109
relative risk of infection in PrP genotype *j* (r_j)												
ARR/ARR†	0	-	0.005	0	-	0.005	0	-	0.001	0	-	0.001
ARR/AHQ†	0	-	0.016	0	-	0.016	0	-	0.004	0	-	0.004
ARR/ARH†	0	-	0.052	0	-	0.054	0	-	0.005	0	-	0.006
ARR/ARQ	0.002	0.000	0.008	0.002	0.000	0.009	0.001	0.000	0.003	0.001	0.000	0.003
AHQ/AHQ	0.093	0.005	0.440	0.089	0.005	0.408	0.033	0.002	0.156	0.035	0.002	0.158
AHQ/ARH†	0	-	0.034	0	-	0.038	0	-	0.003	0	-	0.004
AHQ/ARQ	0.261	0.122	0.502	0.239	0.114	0.449	0.091	0.043	0.176	0.092	0.044	0.173
ARH/ARH	0.007	0.001	0.022	0.007	0.001	0.024	0.001	0.000	0.002	0.001	0.000	0.003
ARH/ARQ	0.013	0.004	0.032	0.015	0.004	0.035	0.002	0.001	0.005	0.002	0.001	0.005
ARQ/ARQ	0.343	0.246	0.476	0.359	0.264	0.488	0.098	0.068	0.138	0.110	0.080	0.150
ARR/VRQ	0.592	0.347	0.975	0.526	0.316	0.843	0.373	0.213	0.630	0.332	0.197	0.537
AHQ/VRQ	0.022	0.001	0.102	0.023	0.001	0.105	0.017	0.001	0.077	0.018	0.001	0.081
ARH/VRQ	0.647	0.381	1.065	0.669	0.405	1.065	0.075	0.044	0.122	0.085	0.052	0.135
ARQ/VRQ	0.619	0.460	0.837	0.639	0.487	0.846	0.417	0.300	0.578	0.451	0.340	0.602
VRQ/VRQ‡	1	-	-	1	-	-	1	-	-	1	-	-

† there were no reported cases, fallen stock positives or abattoir positives for this PrP genotype in 2002; ‡ baseline for risk of infection

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